

# APPENDIX A

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Figure 14 is an agarose gel obtained in Example 13 in which amplification from an RNA template was examined.

Figure 15 is a southern blot of the gel obtained in Figure 14.

Figure 16 is a fluorescence spectrum illustrating the results obtained in Example 14 in which the phenomenon of "strand displacement" using ethidium-labeled oligonucleotides in accordance with the present invention was investigated.

Figure 17 is a fluorescence spectrum illustrating the results obtained in Example 15 in which a T7 promoter oligonucleotide 50 mer labeled with ethidium was employed to study its effect on *in vitro* transcription by T7 and T3 polymerases from an IBI 31 plasmid (pIBI 31-BH5-2) and from a BlueScript II plasmid construct (pBSII//HCV).

Figure 18 depicts the polylinker sequences of the IBI 31 plasmid (pIBI 31-BH5-2) and the BlueScript II plasmid construct (pBSII//HCV).

Figure 19 illustrates a nucleic acid construct in which the host promoter serves as an independent nucleic acid production source (the progeny.

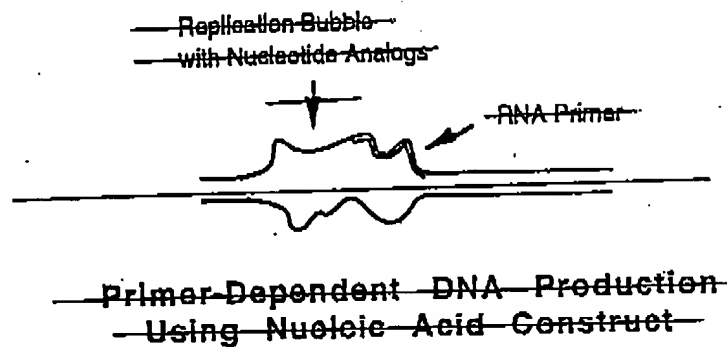
Figure 20 shows a nucleic acid construct in which single-stranded DNA product is made having a hairpin loop and which is useful for forming double-stranded product.

Figure 21 depicts a nucleic acid construct for producing double-stranded product by covalently linking two constructs that make complementary DNA strands.

Figure 22 illustrates a nucleic acid construct containing a polylinker region into which a desirable sequence can be cloned. The result of such a construct is a transient accumulation of gene expression within the cell to deliver sense, antisense, protein or any other gene product into the target cell.

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promoter sites such as the host promoter therefore serving as an independent nucleic acid production source (the progeny).

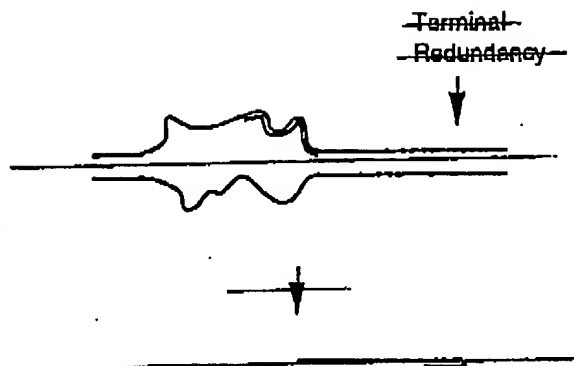


See Figure 19 for an illustration of such a nucleic acid construct in which the host promoter serves as an independent nucleic acid production source (the progeny).

The replication of this structure could result in the production of one strand of DNA product. Several alternative events may occur allowing for the formation of a second complementary strand. For example, a terminal loop could be inserted at the end of the construct such that the single-stranded product will code for the synthesis of the complementary strand using the repair enzyme. Constructs can be made that produce single-stranded DNA product that has a hairpin loop and therefore, can be used to form a double-stranded product.

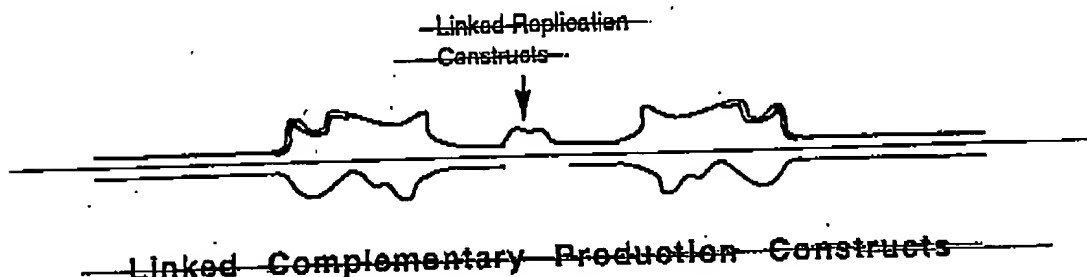
Alternatively, constructs can be formed that produce nucleic acid in both polarities.

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~~Hairpin Product~~

See Figure 20 for a nucleic acid construct in which double-stranded DNA product is made having a hairpin loop and which is useful for forming double-stranded product.

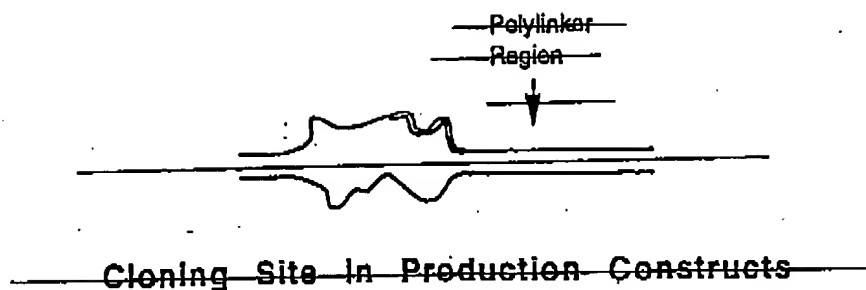
An alternative approach to the production of double-stranded product is to covalently link two constructs that make complementary DNA strands.



See Figure 21 for a depiction of a nucleic acid construct for producing double-stranded product by covalently linking two constructs that make complementary DNA strands.

The construct can be made to contain a poly linker region into which any sequence can be cloned. The result will be a transient accumulation of expressing genes within the cell to deliver sense, antisense or protein or any other gene product into the target cell.

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See Figure 22 illustrates a nucleic acid construct containing a polylinker region into which a desirable sequence can be cloned. The result of such a construct is a transient accumulation of gene expression within the cell to deliver sense, antisense, protein or any other gene product into the target cell.

Other processes within the invention herein described apply to the production of more than one copy of functional genes or antisense DNA or RNA in target cells.

#### Production of Primers

Primers can be produced by several methods. Single-stranded oligonucleotides in the range from between from about 5 to about 100 bases long, and preferably between from about 10 to about 40, and more preferably, between from about 8 to about 20 nucleotides. These ranges may further vary with optimally between from about 13 to about 30 for bacterial nucleic acid, and optimally between from about 17 to about 35 for eukaryote nucleotides would appear to be appropriate for most applications although it may be desirable in some or numerous instances to vary the length of the primers. Oligonucleotide primers can be most conveniently produced by automated chemical methods. In this way modified bases can be introduced. Manual methods can be used and may in some cases be used in combination with automated methods. All of these methods and automation are known and available in the art.